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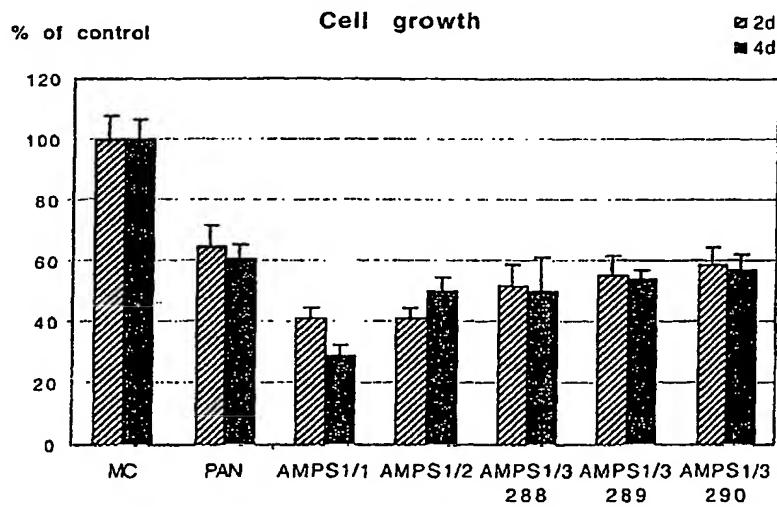
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(54) Title: POLYMERIC COMPOSITION, MATERIAL PRODUCED THEREOF AND THEIR APPLICATIONS



WO 2004/024815 A1

(57) Abstract: The invention relates to a novel polymer composition comprising at least one copolymer constituted of acrylic (AC) monomer units and of at least one water-soluble comonomer unit, which is preferably 2-acrylamido-2-methyl-propane sulfonic acid (AMPS). The composition is used to produce materials, such as membranes, film or surface coatings, that are suitable as highly tissue compatible supports for epithelial cells, such as hepatocytes. The materials are suitable to be used in a bioartificial liver, where they work as separating membrane and carrier for adhesion-dependent tissue cells. As an example it is described how selectively permeable membranes are formed by a phase inversion process from these acrylic copolymers with varying content of 2-acrylamido-2-methyl-propane sulfonic acid (AMPS) and how they interact with liver cells to construct a biohybrid organ for liver replacement.

Polymeric composition, material produced thereof and their applications

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The invention relates to a novel polymeric composition, a method for its production, and its use for the production of materials to be employed in medical or biological applications, particularly for biohybrid or bioartificial organs, involving a direct contact of 10 the material with fluids and/or cells and/or tissues.

Biohybrid or bioartificial organs are designed to support or temporarily replace human organs with malfunctions. The hybrid usually comprises membranes having "passive" separation, filter and carrier functions and also living organ cells having "active" transport 15 and secretion functions.

In artificial organs like the artificial kidney so far only the passive separation and filter function of the organ is replaced with the help of specialised polymeric membranes. However, in the healthy kidney the filtration process is followed by an active re-20 adsorption process realised by the kidney epithelium. In this process, ingredients of the primary urine and water are transported against a concentration gradient from the primary urine back to the blood. Furthermore the kidney epithelium possesses an endocrine function responsible for release of erythropoietin, vitamin D hormones, angiotensins II, kinins and renins contributing to the homeostasis of the organism.

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Analogue situations exist for the liver being one of the most important endocrine gland of the organism secreting hormones and other proteins (albumins, α - und β -globulins, fibrinogen, prothrombin, clotting factors) with storage function for glycogen, proteins, vitamins, beside its detoxifying function. It is clear that with the help of pore membranes 30 only passive filtration and sorptive functions of this organ responsible for detoxification can be achieved and used for therapeutic purposes.

Therefore the immobilisation of organ cells in so-called biohybrid organs was proposed to use the active transport and secretion function of viable cells for supporting or 35 replacing the functions of diseased organs. Until now the achieved results are of little therapeutic value because on the one hand the viability of cells can not be maintained

for the necessary time period and on the other hand the functionality of the immobilised cells achieved so far does not correspond to the functionality of the natural organ.

One of the main reasons of this unsatisfactory functionality originates from the membrane in terms of being a carrier of anchorage dependent cells. Corresponding to the present knowledge the membrane carrier should possess the following properties for optimal functionality of the cells:

1. In general there are insufficient number of primary cells from the patient's own organ available. Therefore donor cells of human or animal origin have to be used in biohybrid organs. In every case a shielding of the patient from allogenic or xenogenic cells in the bioreactor is necessary to prevent activation of the host immune system. This immune isolation limits the maximum pore size of the membrane. Antibodies, complement factors, viruses etc. should not pass through the membrane. On the other hand the permeability of the membrane should permit the exchange of substances necessary for supply to the cells (nutrients, some proteins, such as albumin, gases, toxins to be removed) and removal of the products of the cells (cell metabolites, specific proteins, etc.).

2. Organ cells like hepatocytes are adhesion-dependent and require an extracellular matrix at the contact sites to the membrane where they are anchored. Only in this way do they maintain viable over a longer time period, develop their normal morphology and function and organise in polar, tissue-like structures. Hence, the membrane must allow the moderate adsorption of extracellular matrix proteins without major conformational changes and allow their subsequent structural organisation. Consequently, the membrane surface contacting the tissue cells has to fulfil extreme requirements to be tissue compatible.

Depending on the organ to be supported or replaced and the requirements stated in paragraph 1 and 2 the protein adsorption capacity of the membrane has a crucial importance. Endocrine organs produce proteins that have to be delivered into the blood stream without major adsorption during passage through the membranes to fulfil their function in the human body. Thus, the membrane may have only a moderate adsorption capacity for proteins.

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It is therefore an object of the present invention to provide a composition and a material made of the composition, both suitable for being used in applications involving direct

contact with cells, especially tissue cells. The material made from the composition shall support moderate adsorption of the cells and support their natural functions. Moreover, the composition should be suitable for preparing membranes with pore sizes that enable exchange of substances, which are necessary for the supply of the cells. At the same 5 time, the pore size should ensure an efficient immune isolation to shield a patient from the allogeneic or xenogeneic cells attached to the membrane.

This and other objects are accomplished by a polymeric composition comprising at least one acrylic copolymer constituted of 90 to 99 mole-% of acrylic (AC) monomer units and 10 of at least one water-soluble comonomer unit.

Furthermore this and other objects are accomplished by a polymeric composition comprising at least one acrylonitrile copolymer constituted of 90 to 99 mole-% of acrylonitrile (AN) monomer units and of at least one water-soluble comonomer unit.

15 The comonomer is advantageously an ionic monomer, particularly an anionic monomer. According to a highly preferred embodiment 2-acrylamido-2-methyl-propane sulfonic acid (AMPS) is used as comonomer. Molar contents of AMPS in the copolymer of 1 to 10 mole-%, particularly of 3 to 5 mole-%, have been shown to be favourable with respect 20 to the desired product characteristics as outlined above.

The polymeric composition according to the present invention shows high biocompatibility for many cells, including tissue cells, particularly epithelial cells. Therefore, the composition is suitable for the production of biomaterials, such as membranes, films or 25 coatings, which can be used in medical or biological applications involving direct contact of the material with body fluids and/or cells and/or tissues, particularly with hepatocytes. Cells that are immobilised at the material can easily be fed by a fluid stream, which supplies the cells with nutrients thereby enabling exchange of substances into the cells and outside the cells.

30 It has been shown that materials, produced of the composition can advantageously be used in biohybrid or bioartificial organs, where they work as selectively permeable separating membrane and carrier for adhesion-dependent tissue cells, such as liver, pancreas, lung cells. As shown by experiments on liver cells immobilised on membranes 35 according to the invention, these cells maintain a high degree of natural cytochrome dependent testosterone and ethoxresorufin metabolism and also express glutathione-S-transferase.

Due to their poor cytotoxicity and low affinity for protein adsorption, materials made up of the polymeric composition can also be used for medical applications within the body. For example they may be applied for implants or biosensors.

- 5 The composition can easily be produced by radical copolymerisation of AC, particularly of AC, and at least one water-soluble comonomer in presence of a suitable radical initiator, such as ammonium peroxodisulfate, all dissolved in an aprotic solvent, such as dimethylformamide. To obtain the appropriate product characteristics the reaction is preferably carried out at a total concentration of monomers in the reaction solution in the
10 range of 1 to 5 mole/l, particularly of about 4 mole/l, and a concentration of the radical initiator of about 0,4 to 4 mmole/l.

- Membranes, preferably having a rate of water flux through the membrane in the range of 1 to 10 l/m²hkPa, particularly of about 2 l/m²hkPa, and a cut-off in the range of 150 to
15 1,000 kDa, particularly of 200 to 600 kDa, even more particularly of 300 to 400 kDa are favourably obtained by a phase-inversion process from the copolymers according to the invention. In detail, a casting solution, which contains a polymeric composition at a solid content of for example 15 to 25 weight-% dissolved in a suitable solvent, is prepared. Then the solution is casted on a support or extruded through a suitable nozzle, particularly of the so-called tube-in-orifice type; and the cast or the extruded solution is coagulated by a wet or wet-dry-wet process in a coagulation bath to form an asymmetric membrane. Preferably, the membrane is subsequently subjected to a wet post-treatment in water or steam.
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25 Other preferred embodiments of the invention are disclosed in the dependent claims.

The invention is described in more detail by way of examples and the following figures:

- 30 Figure 1 Cytotoxicity test of membranes prepared according to examples 3 and 4,
Figure 2 MTT test of 3T3 fibroblast growth on membranes according to examples 3 and 4 (control – MC).

Example 1: Polymer synthesis

The copolymers according to the present invention generally comprise poly-acrylonitrile (PAN) derivatives functionalised by the anionic comonomer 2-acrylamido-2-methyl-
5 propane sulfonic acid (AMPS).

The introduction of the functionality was achieved by means of free-radical random copolymerisation techniques to yield copolymers having well adjusted composition ratios and good membrane forming properties. Homopolymerised PAN was included in the
10 studies as reference material.

Before use, AN basic monomer (MERCK, >99 % purity) was inert fractional distilled. AMPS comonomer (ALDRICH, 98 % pur.) was applied without further purification. Dimethylformamide (DMF, ALDRICH, 99,8 % pur.) was inert vacuum distilled at 40
15 mbar/b.p.60°C before application as aprotic solvent in polymer synthesis. Free radical initiators 2,2'-azoisobutyronitrile (AIBN, recrystallised twice from water at 30°C) or ammonium peroxidisulfate (APS, fractional crystallised from ethanol at 40°C) were used in homo- and copolymerisations, respectively.

20 Solution polymerisation initiated by free radicals was carried out as an upscaling procedure (5 l batch size) using a conventional five-necked double-walled jacket glass reactor with an outer thermostating. From the recipe ingredients, first the main portion of aprotic DMF solvent was filled into the reactor and heated up to the desired temperature under continuous stirring (150 r.p.m.) in an inert atmosphere. Subsequently, AN and the
25 comonomer and finally the initiator, dissolved in the remaining DMF, were added into the batch. The reaction time was individually regulated from 360 min (PAN) to 1800 min (AMPS) to obtain the required conversions. In the case of APS initiated copolymer series, the polymerisation temperature was adjusted to 45°C, whereas in the case of AIBN initiated AN homopolymerisation the temperature was 54°C as found by kinetic
30 calculations for adjusting the homo- and copolymer molecular weights.

Because of initiator differentiation it can be assumed definitely that the PAN reference obtained with AIBN is free of any functionalities, even in initiator terminal position.

35 The applied total monomer concentration of $[M] = 3,8 \text{ mole/l}$ for PAN and $5,0 \text{ mole/l}$ for AMPS copolymer includes well graduated molar ratios of the comonomer in the feed, ranging from zero for PAN reference up to 5,0 mole-% for the AMPS enriched feed.

The total monomer concentrations and initiator concentrations of 8 mmole/l (AIBN for PAN) or 4 mmole/l (APS for copolymer series) were balanced in order to obtain number average molecular weights higher than 30,000 g/mole for obtaining membrane forming polymers suitable for the required ultrafiltration pore structure.

After the end of reaction, polymer solutions were poured into a large excess of ethanol used as precipitant. The precipitated polymer was filtered, manifold washed and rinsed with dehydrated ethanol, which was predried on air before exhaustive vacuum drying, until constant weight was reached.

Example 2: Polymer characteristics

- The polymers synthesised from AN and AMPS according to example 1 were characterised for copolymer composition by elementary analysis and number average molecular weight by membrane osmometry. PAN was used as reference material. The results of this analysis are shown in Table 1.
- Table 1: Polymer characteristics. $x_{\text{AMPS, Pol.}}$ = comonomer concentration in copolymer from N-elementary analysis, $M_{n,\text{Osm.}}$ = molecular weight of copolymer measured in 0,1 n NaNO_3/DMF -electrolyte solution.

Polymerisation pattern	Abbreviation	Polymer characteristics	
		$X_{\text{AMPS, Pol.}}$ [mole %]	$M_{n,\text{Osm.}}$ [g/mole $\times 10^{-3}$]
PAN	PAN	-	48.5
P(AN/AMPS)1/1	AMPS1/1	2.9	33.5
P(AN/AMPS)1/2	AMPS1/2	3.2	32.3
P(AN/AMPS)1/3	AMPS1/3	2.9	45.3
P(AN/AMPS)2/1	AMPS2/1	5.2	57.3

Example 3: Membrane formation

Membranes were formed by a phase inversion process from a solution of the polymers according to examples 1 and 2 in dimethylformamide. The polymer concentration varied

from 10 to 22,5 wt-%. The solution was cast on a non-woven support (AMPS1/1, 1/2 and 2/1) or directly on a stainless steel band with a casting slit of 300 µm and a drawing speed of 2 to 4 m/min. After passage through a water precipitation bath at 5 °C or room temperature membranes were tempered in water for 10 min (Table 2).

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Table 2: Membrane formation data.

Membrane	Polymer concentration in solution [wt-%]	Temperature of polymer solution	Precipitation bath	Tempering
PAN	15	1-2 °C	5 °C	10 min 90 °C
AMPS1/1	22,5	RT	RT	10 min 65 °C
AMPS1/2	22,5	RT	RT	10 min 65 °C
AMPS2/1	22,5	RT	RT	10 min 65 °C

The formation of membranes from AMPS1/3 with polymer concentration less than 15% for getting a larger pore structure was not manageable because the material is highly 10 swellable. Hence blend membranes from PAN and AMPS1/3 were formed with varying pore structures. It was assumed that PAN builds a connected polymer network surrounded by the copolymer AMPS1/3. Data on this membrane formation process are given in Table 3.

15 Table 3: Blend membrane formation data.

Membrane	Polymer concentration in solution [wt %]	Temperature of polymer solution	Precipitation bath	Tempering
PAN/AMPS1/3 (1:1) 288	15	RT	RT	10 min 50 °C
PAN/AMPS1/3 (1:1) 289	10	RT	RT	10 min 50 °C
PAN/AMPS1/3 (3:1) 290	10	RT	RT	10 min 50 °C

Example 4: Membrane sieving characteristics

20 4.1 Water permeability and contact angles

The membranes formed according to example 3 had water permeabilities and water contact angles as given in Table 4. Water fluxes of the membranes were determined in

an ultrafiltration device – a Berghoff stirred cell – with distilled water. Water fluxes of AMPS membranes were less than those of PAN membranes mainly due to the higher polymer concentration for AMPS membrane formation resulting in a more dense structure. Compared to PAN, for the AMPS copolymers significant differences in the 5 advancing (wetting) and receding (dewetting) water contact angles were measured indicating more hydrophilic surfaces of the latter materials.

Table 4: Water permeabilities and water contact angles of membranes.

Membrane	Water flux [l/m ² hkPa]	Water contact angle advancing [°]	receding [°]
PAN	4.02	59.5	43.0
AMPS1/1	0.022	47.3	13.8
AMPS1/2	0.020	54.4	17.1
AMPS2/1	0.020	30.5	15.6
PAN/AMPS1/3 (1:1) 288	0.200	0	0
PAN/AMPS1/3 (1:1) 289	0.838	0	0
PAN/AMPS1/3 (3:1) 290	2.205	0	0

10 The water fluxes of AMPS blend membranes increase with lower polymer concentration and higher PAN content in the casting solution. The separation parameters of PAN/AMPS1/3 290 are close to the requirements of a membrane for nutrition supply and waste disposal for tissue cells (water flux about 2 l/m²hkPa) and can be adjusted towards desired values.

15 For the active surfaces of the blend membranes advancing and receding water contact angles of 0° were found indicating highly hydrophilic surfaces.

4.2 Membrane performance

20 To demonstrate the ability to change selectively porosity and transport properties during membrane formation process, AMPS blend membranes with different polymer concentrations and compositions in casting solutions were formed according to examples 1 to 3.

25 Sieving properties of membranes were determined in a Berghoff stirred cell with distilled water and a solution of a test substance mixture (dextrans with molecular weights from

650 to 215.000). Molecular size distributions of dextrans in permeates, in retentates and in the original solution were determined using gel permeation chromatography (GPC). From the results of GPC the average pore diameter (D_{50}), the maximal pore diameter (D_{100}) and the corresponding molecular weights (cut-off) were calculated by assuming a
 5 logarithmic normal distribution of the pore size (Table 5).

Table 5: Separation ability and performance characteristics of PAN/AMPS copolymer blend membranes.

Membrane	Pore characteristics			Cut-off [kDa]
	D_{50} [nm]	D_{100} [nm]	σ	
PAN/AMPS1/3 (1:1) 288	3.73	7.84	0.2573	23.2
PAN/AMPS1/3 (1:1) 289	8.73	20.08	0.2889	188
PAN/AMPS1/3 (3:1) 290	20.75	33.56	0.1668	588

10 The separation parameters of PAN/AMPS1/3 (3:1) are close to the requirements of a membrane for immunoisolation (cut-off 300 to 400 kDa) and can be changed towards desired values.

15 Membrane permeability was investigated by measurements of albumin permeability. A 3 mM albumin solution was used as test solution and PBS as buffer on the sink side of a membrane test cell. Samples were taken every 20 min from the sink side and were measured for an increase in albumin concentration by a Lowry assay (Table 6).

Table 6: Permeability for albumin of PAN/AMPS copolymer blend membranes.

Membrane	Membrane permeability k_o [cm/min]
PAN/AMPS1/3 (1:1) 288	$3.3 \times 10^{-5} \pm 2.8 \times 10^{-5}$
PAN/AMPS1/3 (1:1) 289	$10.5 \times 10^{-5} \pm 7.9 \times 10^{-5}$
PAN/AMPS1/3 (3:1) 290	$10.9 \times 10^{-5} \pm 10.4 \times 10^{-5}$

20

Example 5: General biocompatibility – contact with 3T3 fibroblasts

For testing the general biocompatibility of membranes produced according to examples
 25 1 to 4, experiments with 3T3 fibroblast were performed. The cytotoxicity was tested

according to ISO10993-5 with extracts of membranes obtained after 1, 3 and 7 days of incubation of samples in cell culture medium (DMEM). As shown by neutral red test, confluent layers of 3T3 fibroblasts did not show inhibition of viability after 24 hours incubation with these extracts indicating no cytotoxic properties (Figure 1).

5

Membranes prepared according to examples 1 to 4 were tested for biocompatibility after 2 and 4 days of direct contact to 3T3 fibroblasts. Results were compared to a reference material (Millicell® cell culture insert by Millipore). Figure 2 shows growth rates of 3T3 fibroblasts estimated by the MTT assay. Compared to the reference material PAN, copolymer membranes have less favourable surfaces properties for fibroblasts. The first batch (AMPS1/1) yields still significantly lower growth values for these cells.

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Example 6: Membranes in contact with hepatocytes

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6.1. Plating efficiency and maintenance of cytochrome content

20

Primary cultures of hepatocytes are renowned for their phenotypic instability. Moreover, the concentration of total cytochrome P450 and the activities of its isoenzymes are recognised as the most labile functions, extremely sensitive to the culture environment. Thus, hepatocytes were used to assess the maintenance of functions of the primary cultures on membranes according to examples 1 to 4. If the membranes permitted maintenance of this function it is assumed that they were likely to be suitable for supporting most of hepatocyte functions.

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Hepatocytes were isolated from male Sprague-Dawley rats (180-220 g) by collagenase perfusion. Viability of 80-90 % of isolated cells was confirmed by Trypan Blue exclusion. Hepatocytes were seeded at 1.6×10^8 viable cells per membrane (membrane discs 40 mm in diameter) or 3×10^8 viable cells per collagen-coated ($25 \mu\text{g}/\text{cm}^2$ Type I collagen isolated from rat tail tendons) 60 mm diameter polystyrene Petri dishes (for control dishes). Cells were cultured in Chee's medium supplemented with 5 % (v/v) foetal calf serum, in an atmosphere of 5 % CO_2 in air. Four hours after seeding, the cultures were washed to remove unattached cells, and fresh medium was supplied at this time and once more after 24 h. Samples were taken for assessment of the membranes after 48 h. Plating efficiency was determined by measuring total cell protein by the Lowry assay. Cytochrome P450 and b5 were measured by a modified method of Omura and Sato. Results are given in Table 7.

Table 7: Plating efficiency and cytochrome maintenance of hepatocytes cultured for 48 h on the membranes (control = collagen-coated polystyrene Petri dishes, control cell values = 133.42 pmole/mg protein for cytochrome P450 and 155.42 pmole/mg protein for cytochrome b5). Values are means \pm SEM, number of experiments given parentheses.

Membrane	Plating efficiency [%]	Cytochrome maintenance [% of control]	
		Cyt P450	Cyt b5
control	66.4 \pm 16.0 (6)	100	100
PAN	63.2 \pm 12.0 (5)	92.7 \pm 43.2 (6)	109.2 \pm 38.1 (6)
AMPS1/1	66.4 \pm 16.0 (5)	95.3 \pm 16.1 (5)	107.3 \pm 22.8 (5)

6.2 Cytochrome P450 dependent testosterone metabolism and O-deethylation of ethoxresorufin (EROD)

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The biocompatibility of membranes according to examples 1 to 4 in terms of supporting primary cultures of rat hepatocytes was assessed by measuring the cytochrome P450 dependent metabolism of testosterone and ethoxresorufin.

15 Primary rat hepatocytes isolated according to example 6.1 were seeded with a density of 1.6×10^6 cells per membrane (cultures on membrane discs, 40 mm in diameter) and 3×10^6 cells per collagen-coated 60 mm diameter Petri dish as controls. Cells were cultured in Chee's medium with 5 % foetal calf serum. After 48 h in culture, cells on the membranes and controls were incubated with 100 μ M testosterone in serum-free
20 medium for 60 min. Products were analysed by HPLC. In a second test, the cells cultured for 48 h were washed, scraped into sodium phosphate buffer and homogenised using 7 strokes of a motor driven homogeniser. 0.5 ml of the homogenates were incubated with 6 μ M ethoxresorufin in the presence of NAPDH for 10 minutes. Formation of resorufin was measured fluorimetrically.

25

Table 8 demonstrates a high testosterone metabolism found for rat hepatocytes on the AMPS1/1 membrane, although this functionality of liver cells on the membranes according to the invention is less than that on the collagen control. However, the range of hydroxylated metabolites formed on the AMPS1/1 membrane was the best of all

membranes tested. Cells on this membrane formed 6-beta-hydroxy-, 16-alpha-hydroxy, 16-beta-hydroxytestosterone and androstanedione, whereas most of the other membranes including the PAN membrane supported only the conversion of testosterone to androstanedione. Table 9 shows that the EROD activity is higher in cells cultured on 5 PAN or AMPS1/1 membranes than on controls.

Table 8: Total testosterone metabolites formed by cell cultures (n=3) on PAN and AMPS1/1 membranes. Controls = cells cultured on collagen-coated polystyrene Petri dishes.

Membrane	Total testosterone metabolism [nmole/h/mg protein]
Control	5.4 ± 0.2
PAN	1.5 ± 1.5
AMPS1/1	3.4 ± 1.5

10 Table 9: The activity of EROD in cells cultured on PAN and AMPS1/1 membranes (n=3). Controls = cells cultured on collagen-coated polystyrene Petri dishes.

Membrane	EROD activity [pmole resorufin formed/min/mg protein]
Control	0.6 ± 0.1
PAN	2.0 ± 0.2
AMPS1/1	2.2 ± 0.2

6.3 Glutathione-S-transferase (GST)

15 The expression of alpha- and pi-isoenzymes of glutathione-S-transferase (GST) changes with de-differentiation and age of liver cells in culture. With culturing time, alpha-GST expression declines whereas that of pi-GST increases. Expression of both of these GST proteins was detected in cells growing on the membranes according to the 20 present invention by immunoblotting using specific antibodies. Table 10 shows expression of alpha-GST and pi-GST in cells grown on PAN and AMPS1/1 membranes. Alpha-GST was well maintained in cells grown on both membranes. Cells on the AMPS1/1 membrane had particularly low levels of pi-GST suggesting a higher degree of differentiation.

Table 10: Expression of alpha-GST and pi-GST in cells cultured on PAN and AMPS1/1 membranes (n=3). Controls are cells cultured on collagen-coated Petri dishes.

Membranes	Alpha-GST (OD units)	Pi-GST (OD units)
Control	15.9 ± 0.6	1.5 ± 0.8
PAN	33.7 ± 10.4	2.5 ± 1.9
AMPS1/1	24.3 ± 10.0	0.7 ± 0.6

5 6.4 Long-term performance

At day 7 and 16 of culturing hepatocytes on the AMPS1/1 membrane, cells functions were assessed by the EROD assay, by testosterone metabolism and by GST expression. The effect of collagen coating of the AMPS1/1 membrane on the function of 10 the cells was also investigated. As for the Petri dishes control, collagen was coated at 25 µg/cm² on the membrane. The results are shown in Tables 11 and 12 for testosterone metabolism, in Table 13 for the EROD activity and in Table 14 for the GSTs, respectively. Total metabolism of testosterone was less in cells cultured on the membrane than in cells cultured on the Petri dishes at both 7 and 16 days. Collagen 15 coating of the membrane improved the range of metabolites formed by the cells cultured for 7 days on the membrane, but made little difference after 16 days. At day 7, the collagen-coated membrane also supported cells which showed lower EROD activities, but again this effect could not be observed after 16 days. Collagen coating also improved the expression of alpha GST in cells cultured on AMPS1/1 membrane at 7 20 days, but not after 16 days of culture. However, there was also a high level of expression of pi GST in cells after 7 days culture on the collagen-coated membrane, but no pi-GST was detectable after 16 days with collagen coating.

Long term culture of cells on AMPS1/1 membrane showed that this membrane could 25 maintain viable cells metabolising testosterone and ethoxresorufin and expressing a high level of alpha GST and a low level of pi GST isoenzymes for 16 days. Collagen coating of the membrane improved its performance for up to 7 days, but by 16 days collagen coating made little difference to the enzyme activities of the cells cultured on AMPS1/1 membrane.

Table 11: Total testosterone metabolites formed by cells cultured for 7 and 16 days on the AMPS1/1 membrane, with and without collagen coating (n=3). Controls are cells on collagen-coated Petri dishes.

Membrane / culture time	Total testosterone metabolised [nmole/h/mg protein]
Control, 7 d	7.2 ± 0.8
AMPS1/1, 7 d	1.8 ± 1.0
AMPS1/1 collagen, 7 d	3.7 ± 0.8
Control, 16 d	4.4 ± 0.8
AMPS1/1, 16 d	2.1 ± 0.4
AMPS1/1 collagen, 16 d	1.6 ± 0.9

- 5 Table 12: Formation of metabolites from testosterone by cells cultured for 7 and 16 days on the AMPS1/1 membrane with and without collagen coating (n=3). Controls are cells on collagen-coated Petri dishes. Values are means ± SEM, the formation of each metabolite is expressed as a percentage of the total metabolites formed (see Table 11).

Membrane / culture time	6-alpha Hydroxy	6-beta Hydroxy	16-alpha Hydroxy	2-alpha Hydroxy	Androstendione
Control, 7 d	9.3 ± 1.0	29.8 ± 3.1	0	9.5 ± 1.0	51.3 ± 5.0
AMPS1/1, 7 d	4.6 ± 4.6	30.1 ± 15.4	0	0	32.0 ± 16.0
AMPS1/1 collagen, 7 d	7.5 ± 4.0	30.8 ± 2.6	0	3.5 ± 3.5	58.2 ± 6.3
Control, 16 d	3.7 ± 3.7	26.7 ± 1.5	0	6.2 ± 3.1	63.4 ± 4.9
AMPS1/1, 16 d	0	6.8 ± 6.8	0	5.1 ± 5.1	88.3 ± 6.0
AMPS1/1 collagen, 16 d	0	11.8 ± 11.8	3.5 ± 3.5	0	51.3 ± 28.9

- 10 Table 13: EROD activity of cells cultured for 7 and 16 days on the AMPS1/1 membrane with and without collagen coating (n = 3). Controls are cells cultured on collagen-coated Petri dishes. Values are means ± SEM.

Membrane / culture time	EROD activity [pmole resorufin formed/min/mg protein]
Control, 7 d	0.8 ± 0.2
AMPS1/1, 7 d	0.8 ± 0.7
AMPS1/1 collagen, 7 d	0.4 ± 0.3
Control, 16 d	0.3 ± 0.1
AMPS1/1, 16 d	0.2 ± 0.2
AMPS1/1 collagen, 16 d	1.0, 0.9 (n=2)

Table 14: Expression of alpha-GST and pi-GST in cells cultured on AMPS1/1 membrane with and without collagen coating for 7 and 16 days (n=3). Controls are cells cultured on collagen-coated Petri dishes. Values are means ± SEM.

Membrane / culture time	GST-alpha [OD units]	GST-pi [OD units]
Control, 7 d	11.2 ± 0.5	0
AMPS1/1, 7 d	11.9 ± 11.1	1 ± 1
AMPS1/1 collagen, 7 d	24.0 ± 9.7	11.3 ± 2.6
Control, 16 d	9.7 ± 1.3	4.2 ± 3.7
AMPS1/1, 16 d	20.2 ± 3.4	1.6 ± 0.1
AMPS1/1 collagen, 16 d	11.6 ± 5.6	0

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6.5 Morphology

Hepatocytes isolated and cultured according to example 6.1 were seeded on AMPS1/1 membranes according to examples 1 to 4 and incubated up to 20 days. Some of the 10 membranes were coated with collagen before seeding the cells. As control, hepatocytes were also cultured on collagen-coated Petri dishes. The morphology of the hepatocytes on the AMPS1/1 membranes with and without collagen coating was compared with the control. Cell viability was assessed at day 7, 16 and 20 of culture using carboxyfluorescein diacetate (CFDA) and confocal microscopy. Viable cells were 15 maintained at all time points. The membrane according to the present invention supported cell viability as well as the collagen-coated Petri dishes did. On AMPS1/1 membrane cells showed good morphology, with highly developed bile canaliculi.

CLAIMS

- 5 1. A polymeric composition comprising at least one acrylic copolymer constituted of 90 to 99 mole-% of acrylic (AC) monomer units and of at least one water-soluble comonomer unit.
- 10 2. Composition according to claim 1 comprising at least one acrylonitrile copolymer constituted of 90 to 99 mole-% of acrylonitrile (AN) monomer units and of at least one water-soluble comonomer unit.
- 15 3. Composition according to claim 1 or 2, wherein the comonomer is an ionic monomer, particularly an anionic monomer.
- 20 4. Composition according to claim 3, wherein the comonomer is 2-acrylamido-2-methyl-propane sulfonic acid (AMPS).
5. Composition according to claim 3, comprising AMPS with a molar content in the copolymer of 1 to 10 mole-%, particularly of 3 to 5 mole-%.
- 25 6. Method for producing a composition according to claims 1 to 5, comprising copolymerising acrylic (AC) monomers with at least one water-soluble comonomer in presence of a suitable radical initiator in a suitable solvent.
7. Method according to claim 6, comprising copolymerising acrylonitrile (AN) monomers with at least one water-soluble comonomer in presence of a suitable radical initiator in a suitable solvent.
- 30 8. Method according to claim 6 or 7, wherein the radical initiator is ammonium peroxodisulfate (APS).
9. Method according to any one of the preceding claims 6 to 8, wherein a total concentration of monomers in the reaction solution of 1 to 5 mole/l, particularly of about 4 mole/l, and a concentration of the radical initiator of 0,4 to 4 mmole/l, particularly of about 4 mmole/l, is adjusted.

10. Method according to any one of the preceding claims 6 to 9, wherein the solvent is an aprotic solvent, particularly dimethylformamide (DMS).
11. Use of a composition according to claims 1 to 5 for producing materials being used in medical or biological applications involving a direct contact of the material with fluids and/or cells and/or tissues.
12. Use according to claim 11, wherein the material is a membrane, a film or a surface coating.
13. Use according to claim 11 or 12, wherein the material is employed as support for cells, particularly tissue cells, the cells being in contact with a fluid stream, which supplies the cells with nutrients and enables exchange of substances into the cells and outside the cells.
14. Use according to claim 13, wherein the tissue cells are hepatocytes.
15. Use according to any one of preceding claims 11 to 14, wherein the material is used in biohybrid or bioartificial organs.
16. Use according to claim 15, wherein the material is a membrane with immobilised organ cells, such as liver, pancreas, lung cells, and wherein the cells are separated from the fluid stream.
- 25 17. Use according to any of the preceding claims 11 to 14, wherein the material is used for medical applications within the body, such as implants or biosensors.
18. Membrane, film or coating essentially made up of a composition according to any one of claims 1 to 5.
- 30 19. Membrane, film or coating comprising a blend of a composition according to any one of claims 1 to 5 and poly-acrylonitrile (PAN).
20. Membrane according to claim 18 or 19 being formed by a phase-inversion process.

21. Membrane according to any one of preceding claims 18 to 20, wherein the membrane is an asymmetric membrane, comprising an outer dense layer having an average pore size of 1 to 50 nm, particularly of 3 to 20 nm, more particularly of 5 to 12 nm.

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22. Membrane according to any one of preceding claims 18 to 20, comprising a flat or hollow fibre membrane having at least a two-layer cross sectional structure substantially consisting of a dense surface layer and a porous bulk layer having finger-like pores communicating with the dense layer.

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23. Membrane according to any one of preceding claims 18 to 22, having a rate of water flux through the membrane in the range of 1 to 10 l/m²hkPa, particularly of about 2 l/m²hkPa.

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24. Membrane according to any one of preceding claims 18 to 23, having a cut-off in the range of 150 to 1,000 kDa, particularly of 200 to 600 kDa, more particularly of 300 to 400 kDa.

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25. Method for producing a membrane according to any one of preceding claims 18 to 24, comprising the steps of

- (a) preparing a casting solution, which contains a polymeric composition according to claims 1 to 5 dissolved in a suitable solvent;
- (b) casting the solution on a support or extruding the solution through a suitable nozzle; and
- (c) coagulating the cast or the extruded solution in a coagulation bath to form an asymmetric membrane.

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26. Method according to claim 25, wherein the asymmetric membrane is subjected to a wet post-treatment in water or steam.

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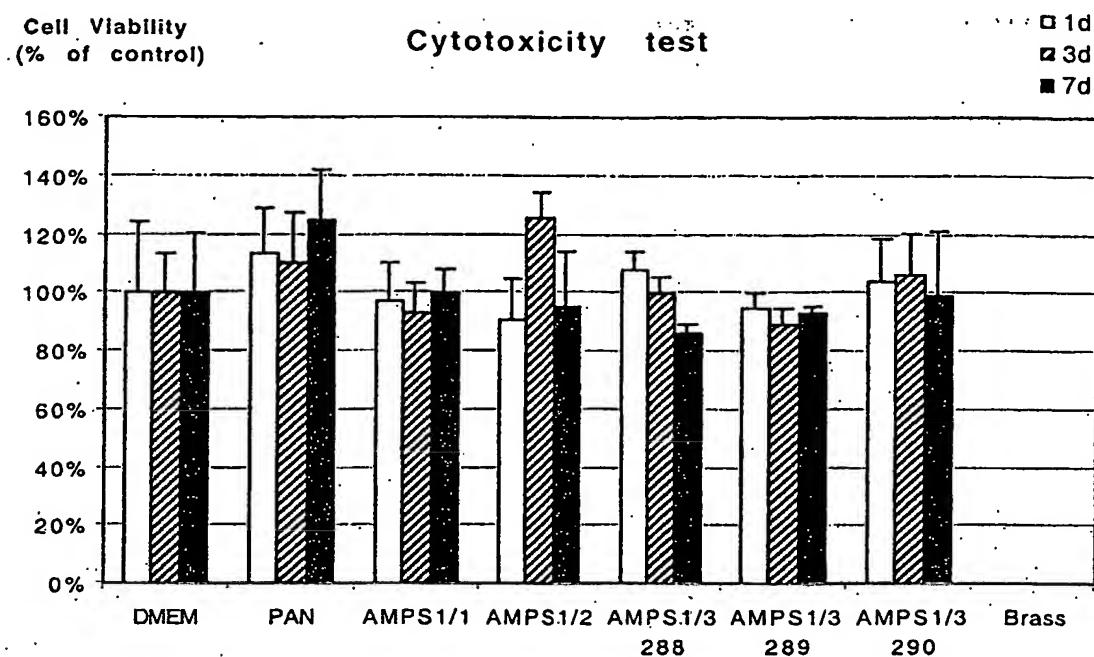
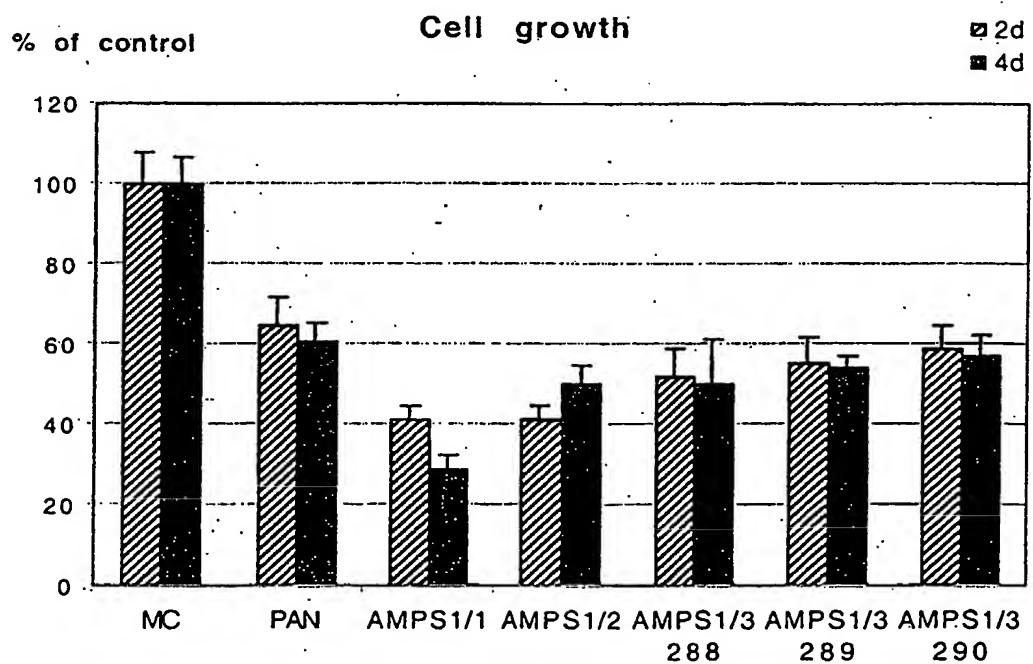
27. Method according to claim 25 or 26, wherein the casting solution is prepared to have a solid content of the polymeric composition of 15 to 25 weight-%.

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28. Method according to any one of preceding claims 25 to 27, wherein the solvent is a polar solvent.

- 19 -

29. Method according to any one of preceding claims 25 to 28, wherein the coagulation is performed by a wet or wet-dry-wet process.
30. Method according to any one of preceding claims 25 to 29, wherein the casting
5 solution is extruded through a tube-in-orifice type nozzle.

Figure 1Figure 2

INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 03/09885

A. CLASSIFICATION OF SUBJECT MATTER
 IPC 7 C08L33/20 C08F220/44 C08F220/58 B01D71/40 A61L27/16

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
 IPC 7 C08L C08F B01D A61L

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data, PAJ

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DE 100 30 307 A (GEESTHACHT GKSS FORSCHUNG) 17 January 2002 (2002-01-17) claims 1-14; examples 1-4 ---	1,2, 6-13, 18-30
X	EP 0 559 902 A (DAICEL CHEM) 15 September 1993 (1993-09-15) claims 1,7; example 1 ---	1-3,6,7, 18 -/-



Further documents are listed in the continuation of box C.



Patent family members are listed in annex.

* Special categories of cited documents :

- "A" document defining the general state of the art which is not considered to be of particular relevance
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Date of the actual completion of the international search

30 January 2004

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INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 03/09885

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	HONIGER J ET AL: "Semiautomatic macroencapsulation of large numbers of porcine hepatocytes by coextrusion with a solution of AN69 polymer" BIOMATERIALS, ELSEVIER SCIENCE PUBLISHERS BV., BARKING, GB, vol. 21, no. 12, June 2000 (2000-06), pages 1269-1274, XP004195837 ISSN: 0142-9612 page 1769, paragraph 1 -----	1-3
A	US 6 130 303 A (RYLES RODERICK GLYN ET AL) 10 October 2000 (2000-10-10) claim 1 -----	4

INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 03/09885

Patent document cited in search report,		Publication date		Patent family member(s)		Publication date
DE 10030307	A	17-01-2002	DE EP	10030307 A1 1177829 A2		17-01-2002 06-02-2002
EP 0559902	A	15-09-1993	JP JP US DE DE EP KR US WO	3171947 B2 5184891 A 5707522 A 69209273 D1 69209273 T2 0559902 A1 9610384 B1 5554292 A 9304769 A1		04-06-2001 27-07-1993 13-01-1998 25-04-1996 01-08-1996 15-09-1993 31-07-1996 10-09-1996 18-03-1993
US 6130303	A	10-10-2000	US	5354481 A		11-10-1994